

Final report of the [YEAR] International Proficiency Testing Scheme (PTS)
for *Mycobacterium avium subspecies paratuberculosis*
antibody detection in serum

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Example

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Introduction

Mycobacterium avium subspecies paratuberculosis (Map) infections in cattle are of major importance in the cattle industry. Detection of antibodies by ELISA is an important tool in many control schemes. A proficiency test can help maintaining the quality of the operational diagnostic systems.

This report contains the results of the [year] International proficiency testing scheme (PTS) for Map (serum) antibody detection in serum, that was organized by the PTS team of Royal GD, the Netherlands.

This PTS is accredited according to the international standard ISO 17043:2010 for providers of proficiency testing schemes.

The goal of this PTS was to determine the performance of individual laboratories for specific tests.

Samples

The sample set consisted of 8 freeze-dried, heat-inactivated (30', 56°C) sera.

The sample numbers #1 - #8, as mentioned in this report, are supplemented with extra information to indicate the origin of the sample. In the presentations of the reported results samples are sorted as indicated in Table 1, supplemented with additional information to indicate the origin of the sample.

Table 1 - Description of the samples

Sample number	Origin	Assigned value
#1	Bovine serum from heavy shedder, 1:50 diluted in negative bovine serum	P
#2	Bovine serum from infected herd	P
#3	Bovine serum from intermediate shedder, 1:10 diluted in negative bovine serum	P
#4	Bovine serum from certified-free herd	N
#5	Bovine serum from heavy shedder, 1:15 diluted in negative bovine serum	P
#6	Bovine serum from heavy shedder, 1:5 diluted in negative bovine serum	P
#7	Bovine serum from infected herd	P
#8	Bovine serum from heavy shedder, 1:25 diluted in negative bovine serum	P

The assigned value is not a sample criterium, it is based on the mean value of 20 measurements, tested in the Royal GD laboratory using an ELISA for the detection of antibodies against Map.

P = all tests were positive

N = all tests were negative

Sample #1 #5, #6 and #8 are dilutions from the same serum sample (diluted in negative serum).

All samples are field samples of herds with a known infection status. Samples from cattle from infected herds were confirmed by faecal culture. Sample #4 is from an animal from a certified Map-free status. Herds with Map-free-certification have proven this free status starting with serology from all cattle of the age of 3 years, followed by negative faecal culture of all adult cattle yearly, for four years. This Map-free status is then to be maintained by two-yearly faecal culture of all adult cattle. The Map-free herd of which samples were included in this PTS had been certified-free for more than six years at the time of sampling.

Homogeneity and stability

The homogeneity and stability of sample sets used in a PTS are based on quantitative results of GD tests.

Homogeneity:

For each sample the coefficient of variation (CV%) is calculated once before the specific sample is used in the PTS, from twenty measurements using the following formula: $(\text{stdev} / \text{mean value}) * 100$.

The CV% is acceptable if lower than the maximum CV% of the test repeatability (CVR).

Stability:

For each sample the coefficient of variation (CV%) is calculated from in total six measurements before and during the participant test period (three different moments), using the following formula: $(\text{stdev} / \text{mean value}) * 100$

The CV% is acceptable if lower than the maximum CV% of the test reproducibility (CVR).

The homogeneity and stability of the samples of this PTS were acceptable.

Homogeneity and stability in comparison with results of the participants:

The homogeneity and stability of the sample set are also assessed by comparing our results with those of the participants, either solely qualitatively or both quantitatively and qualitatively, according to the ISO 13528:2015 guidelines. In the decisive qualitative comparison, an absolute difference of 20% (percentage of positive samples) between the homogeneity samples, stability samples and PTS participants' results is allowed.

For this PTS, only a comparison with the qualitative results of the PTS participants is made.

The between comparison of the homogeneity and stability of samples were acceptable.

In total, 37 laboratories from 16 countries participated in this PTS. Each of the laboratories was given a unique ID-code which was only disclosed to the laboratory itself. In this report, laboratories are identified by their respective ID-codes. The participating laboratories are listed in Table 2. Laboratory names are reported with permission of the participants.

[illegible]

Tests

Reported results included data for ELISAs ($n=38$).
One laboratory reported results for two ELISAs.

ELISA Brand	No of testresults
IDvet ELISA	18
IDEXX ELISA	15
Biosellal EMAP	2
Prionics	1
Indical	1
Other / InHouse	1

Analyses and statistics

A full description of the statistical analysis used in the PTS can be found on the website:
[Statistical analysis used in the PTS](#)

Results

ELISA Results

IDvet ELISA

The IDvet ELISA was used by 18 laboratories. A general overview of the results is presented in the following table 3a.

The qualitative results are based on the cut-off values as indicated by the respective participant.

Tables 4a to 4e and Figures 1 and 2 show the quantitative and qualitative IDvet ELISA results.

Because more than 6 laboratories used this ELISA, z-scores were calculated.

Table 3a - Overview IDvet ELISA results

	Overview IDvet ELISA	+ / +	+ / -	- / -	+ / susp*	- / susp*	susp/susp*	Nd**
#1	heavy shedder, 1:50 diluted	18						
#2	infected herd	18						
#3	intermediate shedder, 1:10 diluted	18						
#4	certified-free herd			18				
#5	heavy shedder, 1:15 diluted	18						
#6	heavy shedder, 1:5 diluted	18						
#7	infected herd	18						
#8	heavy shedder, 1:25 diluted	18						

*susp=suspect

**nd = no qualitative results reported

The z-scores of each laboratory for each sample are presented in Table 4d. Z-score outliers are presented in bold red.

The mean z-score results are presented in the scatter plot diagram (Figure 1).

The trueness of reported ELISA S/P for each of the laboratories varied from -0.59 to 0.35 (Table 4e).

The sR_{within} varied from 0.01 to 0.22 (Table 4e).

The mean sR_{within} was 0.13 S/P unit.

The $sR_{between}$ was 0.37.

IDEXX ELISA

The IDEXX ELISA was used by 15 laboratories. A general overview of the results is presented in the following table 3b.

The qualitative results are based on the cut-off values as indicated by the respective participant.

Tables 5a to 5e and Figures 3 and 4 show the quantitative and qualitative IDEXX ELISA results.

As more than 6 laboratories used this ELISA, z-scores were calculated.

Table 3b - Overview IDEXX ELISA results

	Overview IDEXX ELISA	+ / +	+ / -	- / -	+ /susp*	- / susp*	susp/susp*	Nd**
#1	heavy shedder, 1:50 diluted	15						
#2	infected herd	15						
#3	intermediate shedder, 1:10 diluted	15						
#4	certified-free herd			15				
#5	heavy shedder, 1:15 diluted	15						
#6	heavy shedder, 1:5 diluted	15						
#7	infected herd	15						
#8	heavy shedder, 1:25 diluted	15						

*susp=suspect

**nd = no qualitative results reported

The z-scores of each laboratory for each sample are presented in table 5d. Z-score-outliers are presented in bold red.

The mean z-score results are presented in the scatterplot-diagram (Figure 3).

The trueness of reported ELISA S/Ps for each of the laboratories varied from -0.55 to 1.28 (Table 5e).

Robust within-lab reproducibility varied from 0.01 to 0.10 (table 5e).

The mean sR_{within} was 0.06 S/P units.

The sR_{between} was 0.48.

Other ELISAs

Five laboratories reported results of other ELISAs, from Biosellal EMAP (n=2), Prionics (n=1) Indical (n=1) and Other/InHouse (n=1). A general overview of the results is presented in the following Table 3c.

Tables 6a to 6d show the quantitative and qualitative Other ELISA's results.

Table 3c - Overview Other ELISA's results

	Overview Other ELISA's	+ / +	+ / -	- / -	+ /susp*	- / susp*	susp/susp*	Nd**
#1	heavy shedder, 1:50 diluted	5						
#2	infected herd	5						
#3	intermediate shedder, 1:10 diluted	5						
#4	certified-free herd			5				
#5	heavy shedder, 1:15 diluted	5						
#6	heavy shedder, 1:5 diluted	5						
#7	infected herd	5						
#8	heavy shedder, 1:25 diluted	5						

*susp=suspect

**nd = no qualitative results reported

Because less than 6 laboratories participated with one test system the between-laboratory repeatability (sR_{between}) could not be determined.

The sR_{within} varied from 0.06 till 0.29 S/P (table 6d).

Discussion & Conclusions

All laboratories that participated in this PTS reported positive results for samples from infected, light shedder and high shedder animals, as well as for diluted samples. All participants reported negative results for the serum from a certified-free herd. Therefore, in this PTS, no differences in sensitivity or specificity were observed between laboratories.

Please note:

The objective of this PTS is the evaluation of the laboratory competence in the testing of defined samples by two or more laboratories. Variations may be caused by differences in assay or technique performance as well as laboratory conditions. If your results are not as expected, check your procedures or contact your test kit manufacturer.

Acknowledgements

We would like to thank all participants for their enthusiasm, their quick responses and valuable comments.

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Annex

Table 4a¹ – [year] PTS Map serum IDvet ELISA quantitative results (S/P) per laboratory as reported

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3526	1.137	1.152	2.953	2.438	2.599	2.166	0.039	0.042	2.243	1.819	2.939	2.438	2.953	2.438	1.752	1.543	IDvet Q07
3552	1.336	.	3.077	.	2.797	.	0.053	.	2.359	.	2.964	.	2.978	.	1.927	.	IDvet O35
3613	1.392	1.217	3.401	3.099	3.103	2.545	0.073	0.078	2.523	2.001	3.281	2.986	3.38	3.193	1.937	1.868	IDvet Q35
3647	1.391	1.545	2.75	2.87	2.63	2.672	0.181	0.151	1.492	1.477	1.901	1.777	1.771	1.457	1.307	1.318	IDvet O35
3695	1.01	1.03	2.77	2.63	2.41	2.15	0.07	0.05	1.93	1.68	2.66	2.58	2.79	2.59	1.74	1.34	IDvet O35
3709	0.94	0.94	1.75	1.59	1.71	1.59	0.05	0.03	1.56	1.53	1.78	1.61	1.81	1.61	1.21	1.36	IDvet P61
3712	0.88	1.00	1.39	1.80	1.51	1.67	0.06	0.06	1.41	1.51	1.75	1.95	1.58	1.84	1.39	1.33	IDvet O35
3727	1.338	1.296	2.931	2.772	2.704	2.558	0.042	0.050	2.462	2.253	2.832	2.714	2.898	2.834	1.992	1.810	IDvet O35
3728	1.19	1.235	2.63	2.37	2.28	2.21	0.05	0.095	2.015	1.975	2.66	2.415	2.675	2.45	1.5	1.615	IDvet O35
3758	1.193	1.256	2.1	2.111	2.331	2.062	0.057	0.061	1.922	1.914	2.076	2.095	2.331	2.09	1.761	1.823	IDvet L57
3765	1.267	1.306	2.922	3.220	2.740	2.804	0.068	0.069	2.441	2.256	2.755	2.931	2.778	3.098	1.989	1.777	IDvet O35
3808	1.25	1.24	2.65	2.72	2.54	2.52	0.05	0.06	2.09	2.19	2.63	2.63	2.61	2.69	1.8	1.84	IDvet O35
3861	1.079	1.073	2.868	2.843	2.253	2.246	0.076	0.074	1.758	1.751	2.78	2.758	2.948	2.93	1.503	1.497	IDvet O35
3870	1.49	1.38	2.18	2.5	2.16	2.46	0.04	0.03	2.12	2.36	2.22	2.52	2.18	2.48	2	2.13	IDvet O89
3949	0.921	1.177	3.221	3.113	2.033	2.749	0.139	0.157	2.159	2.157	3.726	3.519	1.315	3.182	1.937	1.858	IDvet I60
3977	1.221	1.171	3.104	2.978	2.67	2.673	0.048	0.059	2.401	2.184	3.132	3.004	3.132	3.004	1.81	1.84	IDvet O35
3987	1.137	1.132	2.399	2.552	2.37	2.473	0.067	0.066	2.06	2.147	2.394	2.455	2.268	2.451	1.608	1.705	IDvet Q07
3994	1.28	1.23	3.09	3.07	2.7	2.7	0.07	0.05	2.15	1.99	3.04	2.99	3.05	3.14	1.94	1.85	IDvet O35

Table 4a² – [year] PTS Map serum IDvet ELISA Cutoff per laboratory as reported

Lab code	ELISA brand / Remarks	Cutoff
3526	IDvet	0,700 (70%)
3552	IDvet	<=60 negative, >=70 positive
3613	IDvet	0.6
3647	IDvet	S/P <=0.6 Neg, 0.6<sp<0.7 doubt, sp>= 0.7 pos
3695	IDvet	70
3709	IDvet	N<=60<=D<=70<=P
3712	IDvet	S/P%<60%=Negative, 60<S/P%<70%=Doubtful, S/P%>70%=Positive
3727	IDvet	
3728	IDvet	0,70
3758	IDvet	
3765	IDvet	
3808	IDvet	Neg =<0.6 Inconclusive >0.6 - =<0.7, Positive >0.7
3861	IDvet	0.6
3870	IDvet	N</=0,6;P>/=0,7
3949	IDvet	70%
3977	IDvet	0.7
3987	IDvet	0,700
3994	IDvet	S/P > 0.7 : positive

Table 4b – [year] PTS Map serum IDvet ELISA quantitative results (S/P) per laboratory as used

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3526	1.14	1.15	2.95	2.44	2.60	2.17	0.04	0.04	2.24	1.82	2.94	2.44	2.95	2.44	1.75	1.54	IDvet Q07
3552	1.34		3.08		2.80		0.05		2.36		2.96		2.98		1.93		IDvet O35
3613	1.39	1.22	3.40	3.10	3.10	2.55	0.07	0.08	2.52	2.00	3.28	2.99	3.38	3.19	1.94	1.87	IDvet Q35
3647	1.39	1.54	2.75	2.87	2.63	2.67	0.18	0.15	1.49	1.48	1.90	1.78	1.77	1.46	1.31	1.32	IDvet O35
3695	1.01	1.03	2.77	2.63	2.41	2.15	0.07	0.05	1.93	1.68	2.66	2.58	2.79	2.59	1.74	1.34	IDvet O35
3709	0.94	0.94	1.75	1.59	1.71	1.59	0.05	0.03	1.56	1.53	1.78	1.61	1.81	1.61	1.21	1.36	IDvet P61
3712	0.88	1.00	1.39	1.80	1.51	1.67	0.06	0.06	1.41	1.51	1.75	1.95	1.58	1.84	1.39	1.33	IDvet O35
3727	1.34	1.30	2.93	2.77	2.70	2.56	0.04	0.05	2.46	2.25	2.83	2.71	2.90	2.83	1.99	1.81	IDvet O35
3728	1.19	1.24	2.63	2.37	2.28	2.21	0.05	0.09	2.02	1.98	2.66	2.41	2.67	2.45	1.50	1.62	IDvet O35
3758	1.19	1.26	2.10	2.11	2.33	2.06	0.06	0.06	1.92	1.91	2.08	2.10	2.33	2.09	1.76	1.82	IDvet L57
3765	1.27	1.31	2.92	3.22	2.74	2.80	0.07	0.07	2.44	2.26	2.75	2.93	2.78	3.10	1.99	1.78	IDvet O35
3808	1.25	1.24	2.65	2.72	2.54	2.52	0.05	0.06	2.09	2.19	2.63	2.63	2.61	2.69	1.80	1.84	IDvet O35
3861	1.08	1.07	2.87	2.84	2.25	2.25	0.08	0.07	1.76	1.75	2.78	2.76	2.95	2.93	1.50	1.50	IDvet O35
3870	1.49	1.38	2.18	2.50	2.16	2.46	0.04	0.03	2.12	2.36	2.22	2.52	2.18	2.48	2.00	2.13	IDvet O89
3949	0.92	1.18	3.22	3.11	2.03	2.75	0.14	0.16	2.16	2.16	3.73	3.52	1.31	3.18	1.94	1.86	IDvet I60
3977	1.22	1.17	3.10	2.98	2.67	2.67	0.05	0.06	2.40	2.18	3.13	3.00	3.13	3.00	1.81	1.84	IDvet O35
3987	1.14	1.13	2.40	2.55	2.37	2.47	0.07	0.07	2.06	2.15	2.39	2.45	2.27	2.45	1.61	1.71	IDvet Q07
3994	1.28	1.23	3.09	3.07	2.70	2.70	0.07	0.05	2.15	1.99	3.04	2.99	3.05	3.14	1.94	1.85	IDvet O35

Table 4c – [year] PTS Map serum IDvet ELISA qualitative results per laboratory as reported

Lab code	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8	ELISA brand / Remarks
	heavy shedder, 1:50 diluted	infected herd	intermediate shedder, 1:10 diluted	certified-free herd	heavy shedder, 1:15 diluted	heavy shedder, 1:5 diluted	infected herd	heavy shedder, 1:25 diluted	
3526	+	+	+	+	+	+	+	+	IDvet Q07
3552	+	+	+	-	+	+	+	+	IDvet O35
3613	+	+	+	-	+	+	+	+	IDvet Q35
3647	+	+	+	-	+	+	+	+	IDvet O35
3695	+	+	+	-	+	+	+	+	IDvet O35
3709	+	+	+	-	+	+	+	+	IDvet P61
3712	+	+	+	-	+	+	+	+	IDvet O35
3727	+	+	+	-	+	+	+	+	IDvet O35
3728	+	+	+	-	+	+	+	+	IDvet O35
3758	+	+	+	-	+	+	+	+	IDvet L57
3765	+	+	+	-	+	+	+	+	IDvet O35
3808	+	+	+	-	+	+	+	+	IDvet O35
3861	+	+	+	-	+	+	+	+	IDvet O35
3870	+	+	+	-	+	+	+	+	IDvet O89
3949	+	+	+	-	+	+	+	+	IDvet I60
3977	+	+	+	-	+	+	+	+	IDvet O35
3987	+	+	+	-	+	+	+	+	IDvet Q07
3994	+	+	+	-	+	+	+	+	IDvet O35

Table 4d – [year] PTS Map serum IDvet ELISA z-scores

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3526	-0.34	-0.25	0.56	-0.59	0.52	-0.89	-1.24	-1.07	0.69	-0.62	0.66	-0.28	0.71	-0.21	0.17	-0.61	IDvet Q07
3552	0.82		0.83		1.16		-0.46		1.04		0.70		0.75		0.82		IDvet O35
3613	1.15	0.13	1.56	0.88	2.16	0.34	0.67	0.96	1.55	-0.06	1.30	0.75	1.47	1.14	0.86	0.60	IDvet Q35
3647	1.14	2.03	0.10	0.37	0.62	0.76	6.76	5.07	-1.62	-1.67	-1.29	-1.52	-1.40	-1.96	-1.48	-1.44	IDvet O35
3695	-1.07	-0.96	0.15	-0.16	-0.10	-0.94	0.50	-0.62	-0.28	-1.05	0.13	-0.02	0.42	0.06	0.13	-1.36	IDvet O35
3709	-1.51	-1.50	-2.12	-2.47	-2.37	-2.75	-0.38	-1.52	-1.41	-1.50	-1.51	-1.84	-1.33	-1.69	-1.83	-1.28	IDvet P61
3712	-1.83	-1.12	-2.94	-2.02	-3.03	-2.52	-0.30	0.10	-1.88	-1.55	-1.58	-1.21	-1.75	-1.28	-1.18	-1.39	IDvet O35
3727	0.83	0.59	0.51	0.15	0.86	0.39	-1.06	-0.64	1.36	0.72	0.46	0.24	0.61	0.50	1.06	0.39	IDvet O35
3728	-0.03	0.23	-0.16	-0.74	-0.52	-0.75	-0.62	1.91	-0.02	-0.14	0.13	-0.33	0.21	-0.19	-0.76	-0.34	IDvet O35
3758	-0.01	0.36	-1.35	-1.32	-0.35	-1.23	-0.23	0.00	-0.30	-0.33	-0.96	-0.93	-0.40	-0.83	0.20	0.43	IDvet L57
3765	0.42	0.64	0.49	1.15	0.98	1.19	0.37	0.43	1.30	0.73	0.31	0.64	0.40	0.97	1.05	0.26	IDvet O35
3808	0.32	0.26	-0.12	0.04	0.33	0.26	-0.62	-0.06	0.22	0.52	0.08	0.08	0.10	0.24	0.35	0.50	IDvet O35
3861	-0.67	-0.71	0.37	0.31	-0.61	-0.63	0.84	0.73	-0.81	-0.83	0.36	0.32	0.70	0.67	-0.75	-0.78	IDvet O35
3870	1.72	1.08	-1.17	-0.45	-0.91	0.07	-1.19	-1.75	0.31	1.05	-0.69	-0.13	-0.67	-0.13	1.09	1.57	IDvet O89
3949	-1.59	-0.10	1.16	0.91	-1.32	1.01	4.41	5.42	0.43	0.42	2.13	1.74	-2.21	1.12	0.86	0.56	IDvet I60
3977	0.15	-0.14	0.89	0.61	0.75	0.76	-0.74	-0.12	1.17	0.50	1.02	0.78	1.03	0.80	0.39	0.50	IDvet O35
3987	-0.34	-0.36	-0.68	-0.34	-0.23	0.11	0.34	0.28	0.12	0.39	-0.37	-0.25	-0.51	-0.19	-0.36	0.00	IDvet Q07
3994	0.50	0.21	0.86	0.82	0.85	0.85	0.50	-0.62	0.40	-0.09	0.85	0.75	0.88	1.04	0.87	0.53	IDvet O35

Table 4e – [year] PTS Map serum IDvet ELISA robust within-lab reproducibility and trueness

Lab code	Mean value (S/P)	trueness (S/P)	Robust within-lab reproducibility sd
3526	1.92	0.01	0.22
3552	2.19	0.28	
3613	2.25	0.35	0.18
3647	1.67	-0.24	0.11
3695	1.84	-0.07	0.14
3709	1.32	-0.59	0.10
3712	1.32	-0.59	0.15
3727	2.09	0.18	0.11
3728	1.84	-0.07	0.13

Mean within-lab reproducibility sd	0.13
Mean between-lab reproducibility sd	0.37

Total mean S/P	1.91
Uncertainty	0.05

Lab code	Mean value (S/P)	trueness (S/P)	Robust within-lab reproducibility sd
3758	1.70	-0.21	0.11
3765	2.15	0.24	0.16
3808	1.97	0.06	0.04
3861	1.90	-0.01	0.01
3870	1.89	-0.02	0.19
3949	2.09	0.18	0.16
3977	2.15	0.24	0.09
3987	1.83	-0.08	0.08
3994	2.15	0.24	0.06

Figure 1 – [year] PTS Map serum IDvet ELISA scatter plot

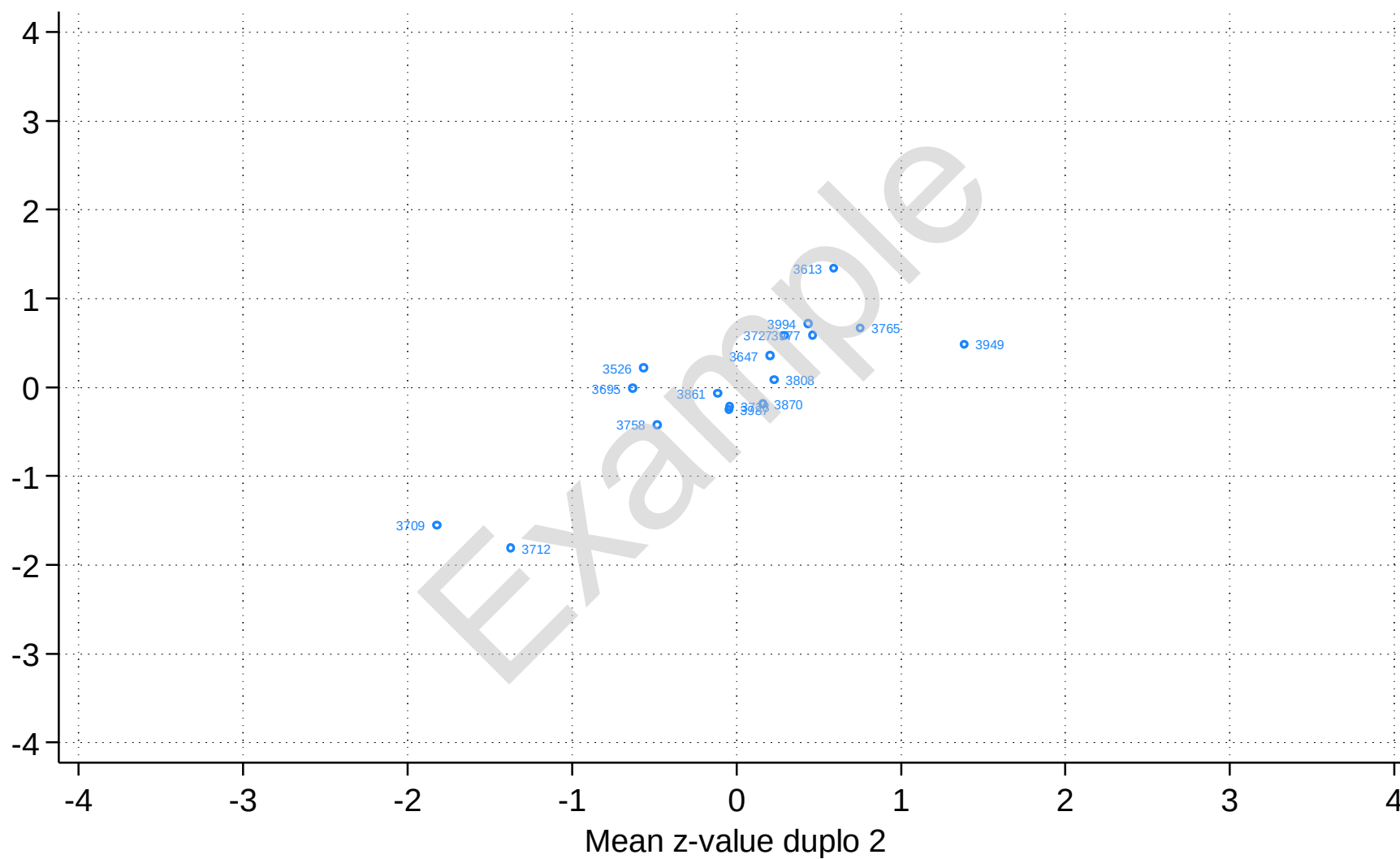


Figure 2 – [year] PTS Map serum IDvet ELISA box plot

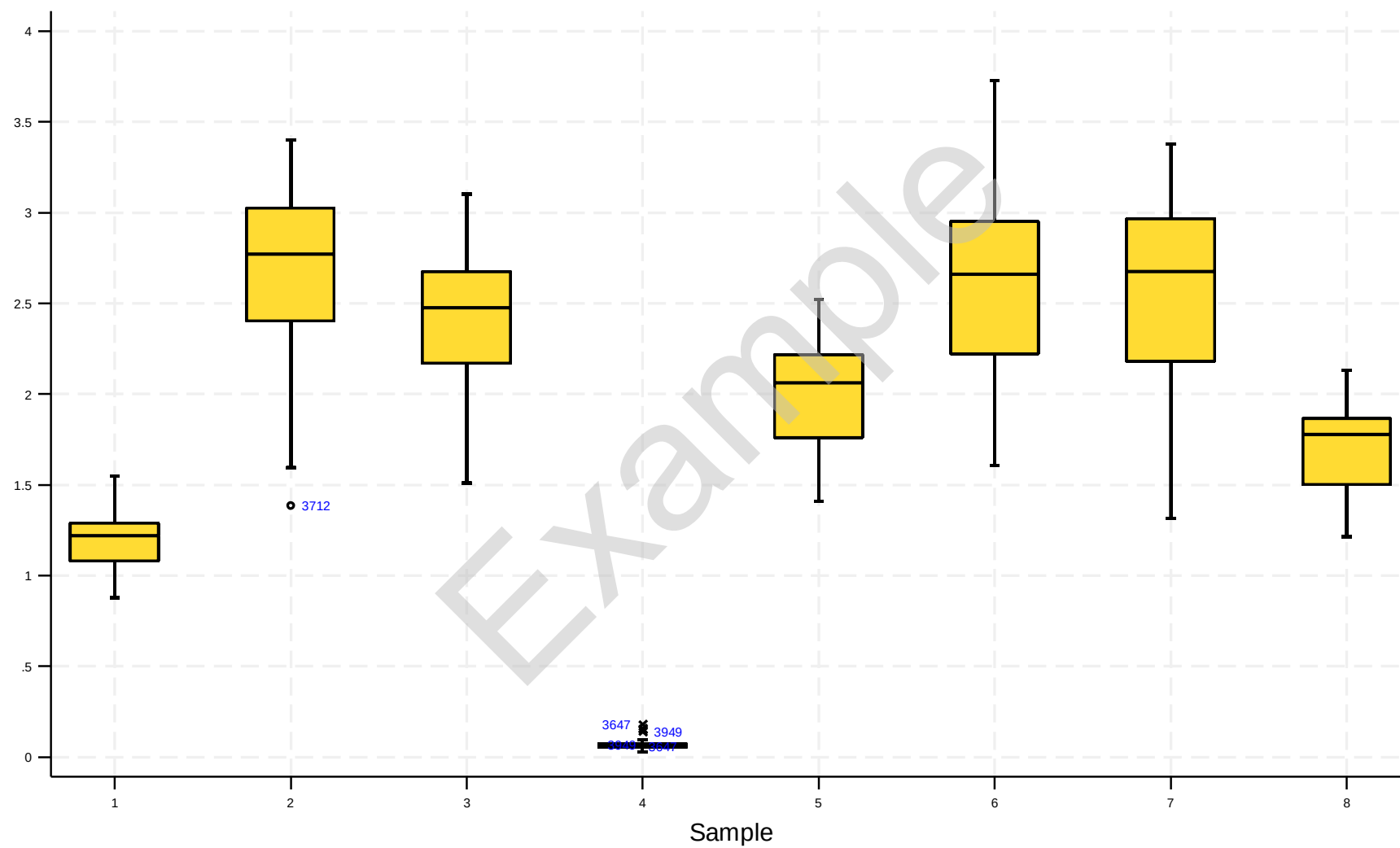


Table 5a¹ – [year] PTS Map serum IDEXX ELISA quantitative results (S/P) per laboratory as reported

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3512	0.95	0.89	1.22	1.22	1.27	1.25	0.04	0.05	1.19	1.20	1.42	1.39	1.50	1.45	1.11	1.12	IDEXX 24071
3527	1.132	1.026	1.78	1.814	1.673	1.761	0.086	0.085	1.594	1.602	2.195	2.176	2.268	2.288	1.421	1.352	IDEXX 23093
3587	1.05	1.01	1.80	1.77	1.74	1.65	0.08	0.08	1.41	1.38	1.99	1.95	2.17	2.16	1.29	1.32	IDEXX 23048
3593	1.805	1.806	3.137	3.122	3.056	3.071	0.133	0.133	3.081	3.065	3.642	3.692	4.104	4.098	2.482	2.476	IDEXX SN24036
3644	1.264	1.215	1.809	1.82	1.793	1.716	0.113	0.111	1.648	1.572	2.031	1.984	2.029	2.033	1.454	1.363	IDEXX 23093
3684	2.36	2.45	3.20	3.64	3.13	3.20	0.09	0.13	3.10	3.32	3.98	3.97	3.90	4.15	2.78	2.78	IDEXX
3707	1.16	1.17	1.93	1.93	1.83	1.84	0.03	0.04	1.64	1.66	2.22	2.25	2.39	2.41	1.46	1.47	IDEXX 24089
3721	1.11	1.06	1.78	1.69	1.64	1.57	0.04	0.05	1.60	1.49	1.98	1.87	2.03	1.94	1.37	1.35	IDEXX 24094
3724	1.02	0.99	1.74	1.73	1.67	1.7	0.05	0.05	1.46	1.48	1.96	1.93	2.14	2.11	1.22	1.26	IDEXX 25013
3809	0.92	0.88	1.24	1.24	1.24	1.22	0.09	0.06	1.17	1.18	1.32	1.38	1.41	1.45	1.1	1.05	IDEXX 25004
3876	1.689	1.324	2.722	2.245	2.534	1.978	0.113	0.098	2.294	1.86	2.57	2.48	3.035	1.172	2.092	1.72	IDEXX 23020
3897	1.145	1.078	2.107	2.281	1.888	2.137	0.043	0.044	1.716	1.712	2.334	2.475	2.299	2.4	1.324	1.496	IDEXX 24094
3900	1.1	0.95	1.61	1.78	1.54	1.69	0.04	0.06	1.55	1.4	1.97	1.96	1.96	1.97	1.23	1.23	IDEXX 24089
3933	2.041	1.629	2.969	2.634	2.599	2.443	0.131	0.159	2.425	2.211	2.246	2.908	3.339	3.15	2.229	1.905	IDEXX
3944	1.06	.	1.78	.	1.63	.	0.05	.	1.64	.	1.99	.	2.13	.	1.26	.	IDEXX 24108

Table 5a² – [year] PTS Map serum IDEXX ELISA cutoff per laboratory as reported

Lab code	ELISA brand / Remarks	Cutoff
3512	IDEXX	
3527	IDEXX	S/P% ≤ 45% ; 45% < S/P% < 55% ; S/P% ≥ 55%
3587	IDEXX	pos.>55, neg.<45
3593	IDEXX	0.55
3644	IDEXX	
3684	IDEXX	
3707	IDEXX	0.55
3721	IDEXX	45-55
3724	IDEXX	45-55
3809	IDEXX	
3876	IDEXX	Neg. ≤45%, pos. ≥55%
3897	IDEXX	NEG<45%DOUTEUX<55%POS
3900	IDEXX	≤0.45 Negative 0.46-0.54 Doubtful ≥0.55 Positive
3933	IDEXX	
3944	IDEXX	45%

Table 5b – [year] PTS Map serum IDEXX ELISA quantitative results (S/P) per laboratory as used

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3512	0.95	0.89	1.22	1.22	1.27	1.25	0.04	0.05	1.19	1.20	1.42	1.39	1.50	1.45	1.11	1.12	IDEXX 24071
3527	1.13	1.03	1.78	1.81	1.67	1.76	0.09	0.09	1.59	1.60	2.19	2.18	2.27	2.29	1.42	1.35	IDEXX 23093
3587	1.05	1.01	1.80	1.77	1.74	1.65	0.08	0.08	1.41	1.38	1.99	1.95	2.17	2.16	1.29	1.32	IDEXX 23048
3593	1.80	1.81	3.14	3.12	3.06	3.07	0.13	0.13	3.08	3.07	3.64	3.69	4.10	4.10	2.48	2.48	IDEXX SN24036
3644	1.26	1.22	1.81	1.82	1.79	1.72	0.11	0.11	1.65	1.57	2.03	1.98	2.03	2.03	1.45	1.36	IDEXX 23093
3684	2.36	2.45	3.20	3.64	3.13	3.20	0.09	0.13	3.10	3.32	3.98	3.97	3.90	4.15	2.78	2.78	IDEXX
3707	1.16	1.16	1.93	1.93	1.83	1.84	0.03	0.04	1.64	1.66	2.22	2.25	2.39	2.41	1.46	1.47	IDEXX 24089
3721	1.11	1.06	1.78	1.69	1.64	1.57	0.04	0.05	1.60	1.49	1.98	1.87	2.03	1.94	1.37	1.35	IDEXX 24094
3724	1.02	0.99	1.74	1.73	1.67	1.70	0.05	0.05	1.46	1.48	1.96	1.93	2.14	2.11	1.22	1.26	IDEXX 25013
3809	0.92	0.88	1.24	1.24	1.24	1.22	0.09	0.06	1.17	1.18	1.32	1.38	1.41	1.45	1.10	1.05	IDEXX 25004
3876	1.69	1.32	2.72	2.24	2.53	1.98	0.11	0.10	2.29	1.86	2.57	2.48	3.04	1.17	2.09	1.72	IDEXX 23020
3897	1.14	1.08	2.11	2.28	1.89	2.14	0.04	0.04	1.72	1.71	2.33	2.47	2.30	2.40	1.32	1.50	IDEXX 24094
3900	1.10	0.95	1.61	1.78	1.54	1.69	0.04	0.06	1.55	1.40	1.97	1.96	1.96	1.97	1.23	1.23	IDEXX 24089
3933	2.04	1.63	2.97	2.63	2.60	2.44	0.13	0.16	2.42	2.21	2.25	2.91	3.34	3.15	2.23	1.90	IDEXX
3944	1.06		1.78		1.63		0.05		1.64		1.99		2.13		1.26		IDEXX 24108

Table 5c – [year] PTS Map serum IDEXX ELISA qualitative results per laboratory as reported

Lab code	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8	ELISA brand / Remarks
	heavy shedder, 1:50 diluted	infected herd	intermediate shedder, 1:10 diluted	certified-free herd	heavy shedder, 1:15 diluted	heavy shedder, 1:5 diluted	infected herd	heavy shedder, 1:25 diluted	
3512	+	+	+	+	+	+	+	+	IDEXX 24071
3527	+	+	+	+	+	+	+	+	IDEXX 23093
3587	+	+	+	+	+	+	+	+	IDEXX 23048
3593	+	+	+	+	+	+	+	+	IDEXX SN24036
3644	+	+	+	+	+	+	+	+	IDEXX 23093
3684	+	+	+	+	+	+	+	+	IDEXX
3707	+	+	+	+	+	+	+	+	IDEXX 24089
3721	+	+	+	+	+	+	+	+	IDEXX 24094
3724	+	+	+	+	+	+	+	+	IDEXX 25013
3809	+	+	+	+	+	+	+	+	IDEXX 25004
3876	+	+	+	+	+	+	+	+	IDEXX 23020
3897	+	+	+	+	+	+	+	+	IDEXX 24094
3900	+	+	+	+	+	+	+	+	IDEXX 24089
3933	+	+	+	+	+	+	+	+	IDEXX
3944	+	+	+	+	+	+	+	+	IDEXX 24108

Table 5d – [year] PTS Map serum IDEXX ELISA z-scores.

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3512	-0.81	-0.99	-1.24	-1.25	-1.18	-1.22	-0.88	-0.73	-1.12	-1.11	-1.42	-1.47	-1.22	-1.30	-0.94	-0.94	IDEXX 24071
3527	-0.26	-0.58	-0.37	-0.31	-0.40	-0.23	0.20	0.18	-0.25	-0.23	0.06	0.02	0.00	0.03	-0.20	-0.37	IDEXX 23093
3587	-0.52	-0.64	-0.33	-0.38	-0.27	-0.44	0.12	0.01	-0.66	-0.73	-0.34	-0.42	-0.16	-0.18	-0.53	-0.45	IDEXX 23048
3593	1.76	1.76	1.77	1.75	2.28	2.31	1.38	1.38	2.99	2.95	2.81	2.91	2.93	2.92	2.36	2.34	IDEXX SN24036
3644	0.13	-0.01	-0.32	-0.30	-0.17	-0.31	0.88	0.83	-0.13	-0.30	-0.25	-0.34	-0.38	-0.38	-0.12	-0.34	IDEXX 23093
3684	3.43	3.68	1.87	2.56	2.41	2.56	0.24	1.29	3.02	3.52	3.46	3.43	2.61	3.00	3.08	3.07	IDEXX
3707	-0.18	-0.16	-0.14	-0.13	-0.09	-0.07	-1.23	-0.87	-0.14	-0.12	0.10	0.16	0.20	0.23	-0.10	-0.07	IDEXX 24089
3721	-0.33	-0.49	-0.37	-0.50	-0.46	-0.60	-0.85	-0.81	-0.23	-0.47	-0.34	-0.56	-0.38	-0.53	-0.32	-0.37	IDEXX 24094
3724	-0.60	-0.69	-0.43	-0.44	-0.40	-0.35	-0.70	-0.70	-0.54	-0.50	-0.39	-0.45	-0.21	-0.25	-0.68	-0.59	IDEXX 25013
3809	-0.90	-1.02	-1.22	-1.22	-1.24	-1.27	0.30	-0.45	-1.17	-1.15	-1.61	-1.49	-1.37	-1.31	-0.97	-1.09	IDEXX 25004
3876	1.41	0.31	1.12	0.37	1.27	0.19	0.88	0.50	1.27	0.33	0.77	0.60	1.22	-1.75	1.42	0.52	IDEXX 23020
3897	-0.22	-0.42	0.15	0.42	0.02	0.50	-0.87	-0.85	0.01	0.01	0.32	0.59	0.05	0.21	-0.43	-0.02	IDEXX 24094
3900	-0.36	-0.81	-0.63	-0.37	-0.66	-0.36	-0.95	-0.45	-0.35	-0.67	-0.37	-0.39	-0.49	-0.48	-0.66	-0.66	IDEXX 24089
3933	2.46	1.23	1.51	0.98	1.39	1.09	1.33	2.03	1.56	1.09	0.15	1.41	1.71	1.41	1.75	0.97	IDEXX
3944	-0.48		-0.37		-0.48		-0.70		-0.15		-0.33		-0.22		-0.59		IDEXX 24108

Table 5e – [year] PTS Map serum IDEXX ELISA robust within-lab reproducibility and trueness

Labcode	Mean value (S/P)	trueness (S/P)	Robust within-lab reproducibility sd
3512	1.08	-0.52	0.02
3527	1.52	-0.09	0.05
3587	1.43	-0.18	0.03
3593	2.68	1.08	0.02
3644	1.50	-0.11	0.04
3684	2.89	1.28	0.08
3707	1.59	-0.02	0.01
3721	1.41	-0.19	0.06

Mean within-lab reproducibility sd	0.06
Mean between-lab reproducibility sd	0.48

Total mean S/P	1.60
Uncertainty	0.06

Labcode	Mean value (S/P)	trueness (S/P)	Robust within-lab reproducibility sd
3724	1.41	-0.20	0.02
3809	1.06	-0.55	0.03
3876	1.87	0.27	0.10
3897	1.65	0.05	0.09
3900	1.38	-0.23	0.08
3933	2.19	0.58	0.10
3944	1.44	-0.16	

Figure 3 – [year] PTS Map serum IDEXX ELISA scatter plot

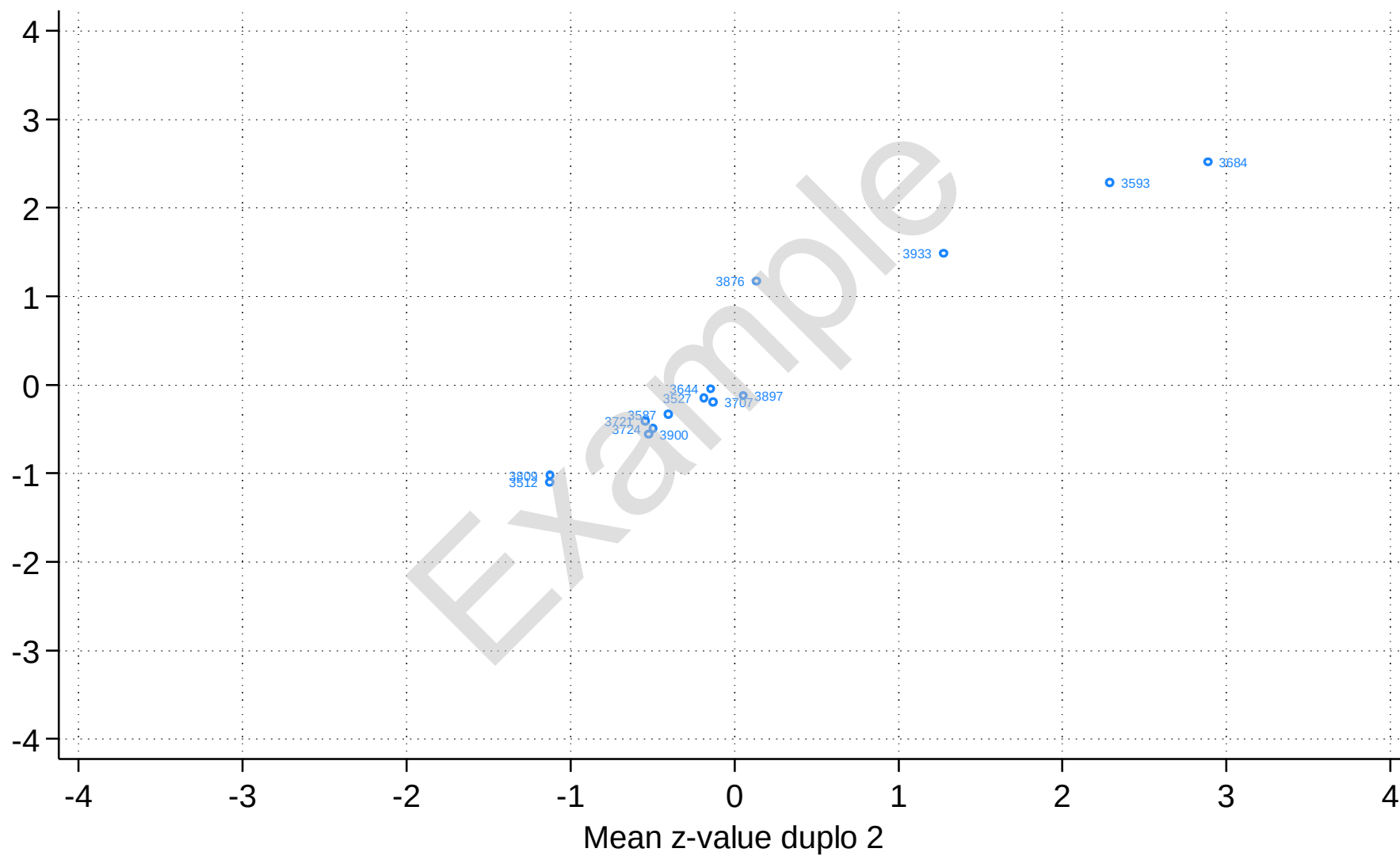


Figure 4 – [year] PTS Map serum IDEXX ELISA box plot

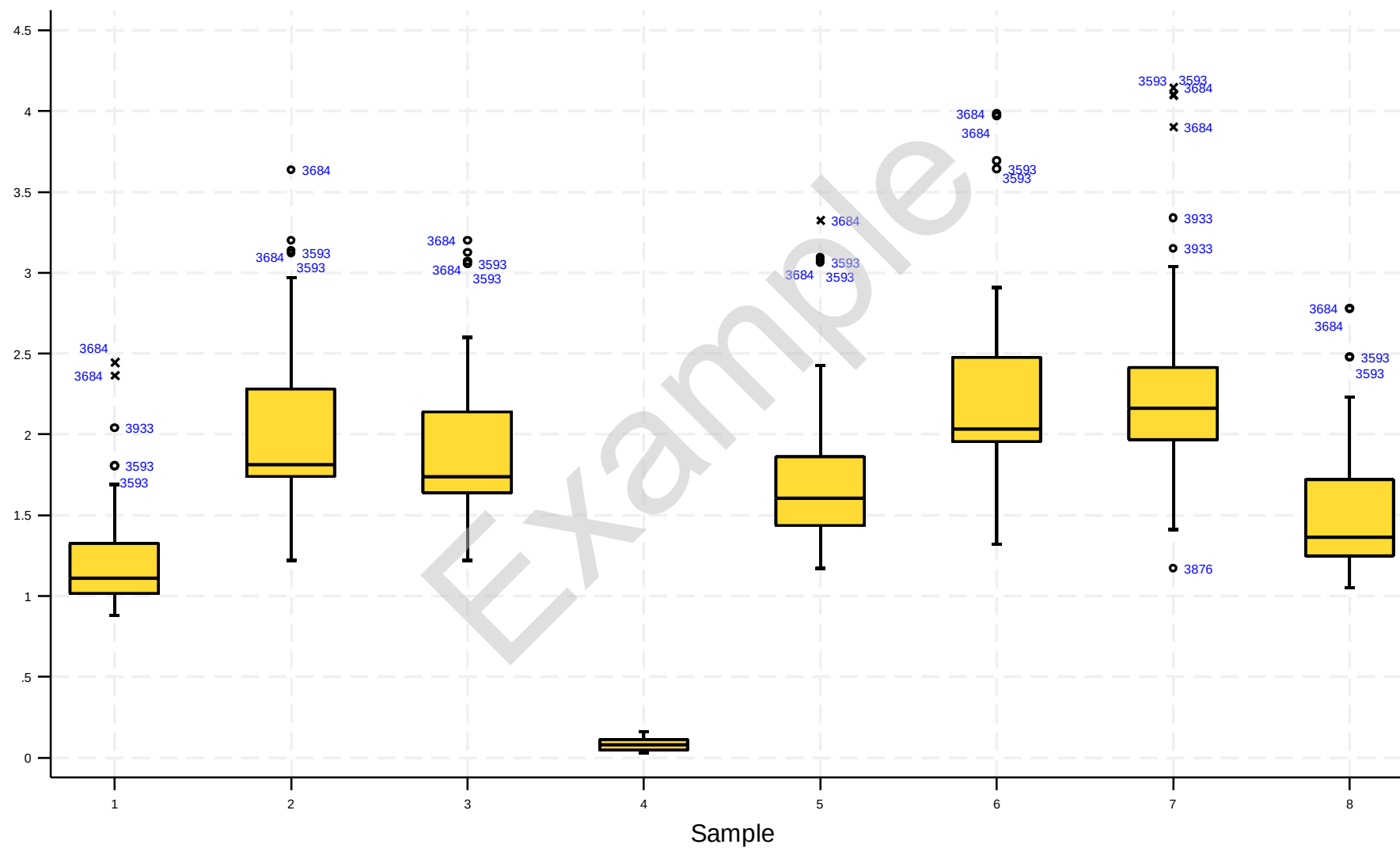


Table 6a¹ – [year] PTS Map serum Other ELISA's quantitative results (S/P) per laboratory as reported

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3602	1.381	1.564	3.222	3.341	2.433	2.452	0.078	0.109	2.389	2.531	3.014	3.294	3.392	3.244	2.106	1.757	Prionics PT241001LA
3612	2.35	2.614	3.513	4.51	2.191	3.2	0.322	0.301	3.144	3.932	4.498	5.16	2.87	4.156	2.522	3.324	240070
3628	0.698	1.012	1.352	1.543	1.112	1.395	0.074	0.07	1.111	1.248	1.562	1.641	1.425	1.667	1.083	1.115	EMAPB-01I
3675	0.705	0.744	2.443	2.512	1.56	1.534	0.033	0.036	1.281	1.416	2.061	2.32	2.924	3.209	1.042	1.208	INDICALF202500006
3959	1.2	1.13	1.652	1.543	1.244	1.181	0.048	0.036	1.568	1.47	2.07	1.956	1.719	1.652	1.399	1.339	BioLisa® kit MAP Ab V2 - EMAPB-01J

Table 6a² – [year] PTS Map serum Other ELISA's cutoff per laboratory as reported

Lab code	ELISA brand / Remarks	Cutoff
3602	Prionics PT241001LA	0.4
3612	240070	POS ≥ 0.55 // NEG ≤ 0.45 // SUSP. = 0.45 - 0.55
3628	EMAPB-01I	0,3
3675	INDICALF202500006	
3959	BioLisa® kit MAP Ab V2 - EMAPB-01J	0,3

Table 6b – [year] PTS Map serum other ELISA's quantitative results (S/P) per laboratory as used

Lab code	Sample #1		Sample #2		Sample #3		Sample #4		Sample #5		Sample #6		Sample #7		Sample #8		ELISA brand / Remarks
	heavy shedder, 1:50 diluted		infected herd		intermediate shedder, 1:10 diluted		certified-free herd		heavy shedder, 1:15 diluted		heavy shedder, 1:5 diluted		infected herd		heavy shedder, 1:25 diluted		
3602	1.38	1.56	3.22	3.34	2.43	2.45	0.08	0.11	2.39	2.53	3.01	3.29	3.39	3.24	2.11	1.76	Prionics PT241001LA
3612	2.35	2.61	3.51	4.51	2.19	3.20	0.32	0.30	3.14	3.93	4.50	5.16	2.87	4.16	2.52	3.32	240070
3628	0.70	1.01	1.35	1.54	1.11	1.39	0.07	0.07	1.11	1.25	1.56	1.64	1.42	1.67	1.08	1.12	EMAPB-01I
3675	0.70	0.74	2.44	2.51	1.56	1.53	0.03	0.04	1.28	1.42	2.06	2.32	2.92	3.21	1.04	1.21	INDICALF202500006
3959	1.20	1.13	1.65	1.54	1.24	1.18	0.05	0.04	1.57	1.47	2.07	1.96	1.72	1.65	1.40	1.34	BioLisa® kit MAP Ab V2 -

Table 6c – [year] PTS Map serum Other ELISA's qualitative results per laboratory as reported

Lab code	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7	Sample #8	ELISA brand / Remarks
	heavy shedder, 1:50 diluted	infected herd	intermediate shedder, 1:10 diluted	certified-free herd	heavy shedder, 1:15 diluted	heavy shedder, 1:5 diluted	infected herd	heavy shedder, 1:25 diluted	
3602	+	+	+	+	+	+	+	+	Prionics PT241001LA
3612	+	+	+	+	+	+	+	+	240070
3628	+	+	+	+	+	+	+	+	EMAPB-01I
3675	+	+	+	+	+	+	+	+	INDICALF202500006
3959	+	+	+	+	+	+	+	+	BioLISA® kit MAP Ab V2 -

Table 6d – [year] PTS Map serum Other ELISA's robust within-lab reproducibility

Lab code	Robust within-lab reproducibility sd
3602	0.15
3612	0.29
3628	0.16
3675	0.13
3959	0.06